UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

184

DATE: February 21, 1980

008819

SUBJECT: Pirimiphos-methyl; PP 9G2154 and FAP 9H5201; Tolerances on Peanuts, Peanut Hulls, Meat, Milk, Poultry, Eggs, and Peanut Oil; and 10182-EUP-RL. Caswell #334B

FROM: John Doherty 2/26/80
Toxicology Branch/HED (TS-769)

TO: F. Sanders, PM #12
Registration Division (TS-767)

Chemistry Branch

Petitioner: ICI Americas, Inc.
Wilmington, Delaware

Recommendations: (Part A; PP 9G2154 and FAP 9H5201)

1. Toxicology Branch does not object to establishment of these subject tolerances if the petitioner agrees to initiate the studies set forth in 2. below and agrees to discontinuance of the tolerances if an undue hazard is indicated by the results of these studies.

2. The following studies are required to be initiated.

   A. With technical pirimiphos-methyl as the test material.

      1. Acute delayed neurotoxicity study in hens

      2. Mutagenesis study (Ames test)

   B. With the formulated product as the test material.

      1. Dermal sensitization study (unless registrant demonstrates product will not come into contact with human skin).

3. Residue Chemistry Branch (see memos by R.B. Perfetti and R.J. Hummel, dated March 29, 1979 and by J. Worthington, dated February 14, 1980 concerned with PP 9G2154 and FAP 9H5201) have expressed several reservations that must be adequately addressed. However, these memos do not raise a question that the residues and metabolites will be of special toxicological concern since data available with rats indicate similar metabolism and metabolities in peanuts and animals. Therefore, no special toxicity studies with metabolites are required at this time to support the present actions.

4. See also the 8-point memo on page 29 of this review.

EPA FORM 1320-6 (REV. 3-76)

1 of 31

See p. 16 under no. 5
p. 18 & 19

Recommendations (Part B, 10182-EUP-RL)

1. The terms of this request for an Experimental Use Permit provide that 715 gal. of ACTELLIC® 7E (5000 lbs. a.i.) be used to treat 125,000 tons (approx.) of peanuts in the states of Alabama (1,000), Florida (800), Georgia (1,600), North Carolina (200), New Mexico (200), South Carolina (200), Texas (800) and Virginia (200). The number of lbs. of active ingredient in each state is in ( ).

   The method of application is by conveyor sprayer to the peanuts as they are being placed into storage. The application rate is 20 ppm or 0.50 lb. a.i./15 tons. The requested EUP time period is for one year from the date of approval. 10 insect pests of stored peanuts will be the proposed targets.

2. Toxicology Branch has no objection to the use of this product in the Experimental Use Permit program if the following item is adequately addressed.

a) Use as directed will not result in a respirable mist or other inhalation hazard. If not, an acute inhalation LC_{50} determination must be provided for the formulated product.

Product to be used for this experimental program:

ACTELLIC® 7E Insecticide (not registered)

Active ingredient: O-[2-(diethylamino)-6-methyl-4-pyrimidinyl] O,O-dimethyl phosphorothioate*	74%
Inerts	26%

The inerts are cleared under 40 CFR 180.1000(c).

* Pirimiphos-Methyl

Summary of Acute Toxicity of ACTELLIC® 7E Insecticide

Test	Results	Tox. Cat.	Core Classification
Acute Oral LD_{50} (rats)	1.3 ml/kg	III	MINIMUM

Acute Dermal LD_{50} (rabbits)	2.52 ml/kg	III	GUIDELINES
Dermal Irritation (rabbits)	Draize = 0.8	IV	GUIDELINES
Eye Irritation (rabbits)	Reversible opacity	II	GUIDELINES

Acute test results support a Toxicity Category II or "WARNING" label based on the eye irritation.

Remarks:

The proposed label must be changed to correct the "Note to Physician" to clearly state that 2-PAM (not P-2AM) or P-25 (pralidoxime) may also be given if available.

The label should also be corrected to say that the product "contains an inhibitor of acetylcholinesterase". The reference to "acetyl choline" poisoning should be deleted.

Review of Acute Studies with Formulation of Pirimiphos-methyl: ACTELLIC® 7E Insecticide.

All studies by Huntingdon Research Centre.

A. Acute Oral Toxicity to rats of ACTELLIC 7E (April 18, 1978; study No. 902?/P211/78)

Groups of 5 male and 5 female Sprague-Dawley, CFY strain rats were fasted prior to being dosed with 0, 0.64, 1.0, 1.6 or 2.5 ml/kg of ACTELLIC 7E.

The overall male and female LD_{50} was determined to be 1.3 (1.1 to 1.6) ml/kg. Female rats were slightly more susceptible than the males. Toxicity Category III.

Terminal autopsy was normal. Deaths occured 28 to 45 hours post dosing. Signs of ChE inhibition developed.

Core Minimum. One discrepancy is that the protocol states that 10 rats were dosed at 4.0 ml/kg, but the results table indicates the highest dose tested was 2.5 ml/kg.

B. Acute Percutaneous Toxicity to Rabbits of ACTELLIC 7E (April 18, 1978; study No. 9022/D212/78)

5 groups of 8 (4 male and 4 female) rabbits prepared as intact (2M and 2F) and abraded (2M and 2F) were dosed with 0, 2.0, 2.5, 3.2 or 4.0 ml/kg of ACTELLIC. Exposure was for 24 hours, and then the rabbits were observed for 14 days.

Signs of toxicity included: lethargy, pilo-erection, increased respiratory rate, abnormal body carriage, body tremors and uncoordinated body movements, increased salivation, diarrhea, and diureses.

After 14 days the combined LD_{50} was 2.52 ml/kg. Autopsy revealed internal congestion. Toxicity Category III.

This test is CORE GUIDELINES.

C. Irritant Effects of ACTELLIC 7E on Rabbit Skin (February 15, 1978; study No. 8862/209D/78)

0.5 ml of ACTELLIC 7E were appliad to the intact and abraded skin areas of New Zealand white rabbits. Application was for 24 hours.

The primary irritation index was calculated to be 0.8. Only very slight erythema and edema developed. Toxicity Category IV.

This test is CORE GUIDELINES.

D. Irritant Effect of ACTELLIC 7E on Rabbit Eye Mucosa (February 6, 1978; study No. 8800/210D/78)

0.1 ml of ACTELLIC 7E were instilled into the conjunctival sac of 9 rabbits. The treated eyes of 6 rabbits were not further rinsed. The eyes of three of the rabbits were irrigated 20-30 seconds after instillation.

Corneal opacity in 5/6 of the unrinsed rabbits' eyes developed. This did not persist to 7 days. The rinsed rabbits' eyes did not develop corneal opacity.

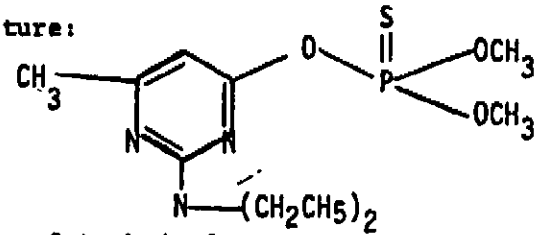
This test is CORE GUIDELINES. Toxicity Category II

Substance Identification:

1. O-[2-(diethylamino)-6-methyl-4-pyrimidinyl]O,O-dimethyl phosphorothioate.

2. Pirimiphos-methyl, ACTELLIC®, PP 511.

3. Structure:



4. Purity of technical material = 94.2%. See reference A3.

5. Other physical/chemical data
   a. specific gravity: 1.157 gm/ml at 30° C.
   b. color/physical state: amber/liquid
   c. Caswell No. 334B
   d. vapor pressure: 1.1×10^{-4} Torr at 30° C
   e. solubility: miscible in all proportions with methanol, ethanol, chloroform, acetone, benzene, toluene and xylene. Less than 5 ppm in water.
   f. chemical class: organophosphate AChE inhibitor.
   g. stability: Technical material is stable for up to 6 months at room temperature. However, mixed with animal food (i.e. rat chow), the half-life is ca.8 or 9 days.

Referenced Petitions: No other existing tolerances.

PP 9G2200, and FAP 9H5217 are currently under consideration and involve the use of pirimiphos-methyl on corn, rice, sorghum and wheat.

SYNOPSIS OF TOXICITY (Technical Material)

Test	Results	CORE Classification
1. Intraperitoneal LD_{50} - rats	800 mg/kg	SUPP.
2. Oral LD_{50} rats (F)	2050 mg/kg	SUPP.
3. Oral LD_{50} mice (M)	1180 mg/kg	SUPP.
4. Oral LD_{50} guinea pigs (F)	1000 - 2000 mg/kg	SUPP.
5. Oral LD_{50} rabbits (M)	1000 - 2000 mg/kg	SUPP.
6. Oral LD_{50} cats	575 - 1150 mg/kg	SUPP.
7. Oral LD_{50} hens	31 - 62 mg/kg	SUPP.
8. Oral LD_{50} dogs	>1500 mg/kg	SUPP.
9. Acute Dermal LD_{50} - rats (F)	>2000 mg/kg	SUPP.
10. Dermal Irritation rats	not irritating	Minimum
11. Eye Irritation - rabbits	not irritating	SUPP.
12. Subacute feeding (14 days, rat) 10 doses orally (gavage) (200 and 400 mg/kg/day)	i. 200 mg/kg/day; weight loss, Hb decrease, other blood and spleen injuries	SUPP.
	ii. 400 mg/kg/day; 65% mortality	SUPP.
13. Subacute dermal (14 days, rabbits)	1000 mg/kg/day, loss in weight, 1 death	SUPP.
14. Inhalation, subacute - rat	3.5 ppm, no toxic signs	SUPP.
15. Skin Sensitization - guinea pig	not a skin sensitizer	SUPP.
16. Subchronic/feeding - dog (90-day)	NOEL < 2 mg/kg/day for RBC ChE inhibition. Systemic NOEL is 2 mg/kg/day. Liver damage at 25 mg/kg/day.	Minimum
17. Oncogenesis study - mouse (18 months) (0, 5, 250, 500 ppm)	No compound related tumors. (NOEL = 5 ppm for cholinesterase)	Minimum
18. Dominant Lethal - mouse (150 mg/kg)	Negative	Minimum
19. Mutagenicity (Ames test)	Mutagenic (?)	SUPP.
20. Teratology - rabbits (0, 1, 16 mg/kg/day)	Not teratogenic	Minimum
21. Reproduction - rats (1) 0, 20, 200 ppm	decreased fertility at 20 ppm	Minimum
22. Reproduction - rats (2) 0, 5, 10, 100 ppm	No effects (on reproduction parameters)	Minimum

23. Human exposure	No effects at 0.25 mg/kg/day for 28 days, orally. Some effects (ChE) at 56 days.	SUPP.
24. Neurotoxicity - hens	Some undefined lesions at 50-60 mg/kg	SUPP.
25. 90-day subchronic oral-rats 0, 8, 80, 360 ppm	ChE inhibition at 80 and 360 ppm. NOEL = 8 ppm (cholinesterase) NOEL = 8 ppm (systemic)	Minimum
26. 2-year rat chronic feeding/oncogenesis study 0, 10, 50, 300 ppm	NOEL = 10 ppm (ChE inhibition) NOEL = 300 ppm (systemic) Not oncogenic.	Minimum
27. 2-year dog chronic feeding 0, 0.5, 2.0, 10.0 mg/kg/day	NOEL < 0.5 mg/kg/day (cholinesterase) (brain ChE is 20% below control) NOEL = 2.0 mg/kg/day (systemic)	GUIDELINES

1. <u>The (acute) toxicity of PP 511 [Pirimiphos-methyl]</u>

ICI Industrial Hygiene Research Laboratories, No. HO/IH/R/276, by D.G. Clark.

A. Intraperitoneal LD_{50}, rats
4M and 4F rats were given intraperitoneal injections of P-M at dose levels of 200, 400, 800 or 1600 mg/kg. An LD_{50} of 800 mg/kg (approximately) for each sex was determined. SUPPLEMENTARY DATA.

B. Acute Oral LD_{50}, Female Rats

Groups of 6 female rats were given P-M by stomach tube.

An oral LD_{50} of 2050 (1850-2270) mg/kg was determined.

SUPPLEMENTARY DATA. No males were used, animals may not have been fasted prior to dosing, no necropsy.

C. Acute Oral LD_{50} - Mice

Groups of 6 male mice were dosed with P-M diluted in propylene glycol. Doses of 800, 1000, 1280, 1600, 2000, 2500 and 3200 were used. An LD_{50} of 1180 (1030-1360) mg/kg was determined.

SUPPLEMENTARY DATA. No females: animals may or may not have been fasted prior to dosing.

D. Acute Oral LD_{50} guinea pig.

Groups of 4 female guinea pigs were given undiluted PP 511 by stomach tube at levels of 500, 1000, 2000 or 4000 mg/kg.

An acute oral LD_{50} was determined to be 1000-2000 mg/kg.
SUPPLEMENTARY DATA.

E. Acute Oral LD50 - rabbits
3 groups of 2 male rabbits were given undiluted PP 511 by stomach tube, doses of 1000, 2000, or 4000 mg/kg were administered.

The acute oral LD_{50} was estimated to be 1000-2000 mg/kg.
SUPPLEMENTARY DATA.

F. Acute Oral LD_{50} - cats

3 groups of either 4, 3 or 2 cats were dosed with 576, 1153 or 2306 mg/kg respectively of P-M.

An LD_{50} of 575-1150 mg/kg was determined.
SUPPLEMENTARY DATA.

G. Acute Oral LD_{50} - hens

5 groups of either 8, 4 or 2 hens were given oral doses of P-M by stomach tube. Doses of 31 (8 hens), 62 (4 hens), 125, 250 and 500 (2 hens) mg/kg were administered.

An LD_{50} of 31-62 mg/kg was determined.
SUPPLEMENTARY DATA.

H. Acute Oral - Dogs

2 male beagle dogs were given a single oral dose of 1500 mg/kg of P-M by stomach tube, a second group was given 750 mg/kg.

No toxic signs developed other than the two dogs at the higher dose vomited slightly within 2 hours after dosing.

SUPPLEMENTARY DATA

I. Acute Dermal Toxicity

A single group of 4 female rats were treated with 2000 mg/kg of P-M (undiluted).

No toxic signs were reported, thus the LD_{50} is greater than 200 mg/kg to rats.

SUPPLEMENTARY DATA

J. Skin Irritation

Four rats were prepared for this test by removal of their fur and 500 mg/kg of the undiluted liquid was applied and left in place for 24 hours. A total of three applications were made in succession, leaving 24 hours between applications.

No signs of irritation developed.

SUPPLEMENTARY DATA. Only 4 rats were used, and no justification for this species was stated. Upgraded to CORE MINIMUM. The three doses applied demonstrate that this product is not a serious dermal irritant.

K. Eye Instillation

One drop of undiluted P-M was placed in the left conjunctival sac of 4 albino rabbits. The right eyes served as controls.

No signs of irritation developed

This test is SUPPLEMENTARY DATA. Insufficient number of animals, insufficient dose, no raw data or method of grading presented.

L. Pharmacological Studies (following an oral dose)

A group of 36 male rats were given a single oral dose of 1450 mg/kg (ca.70% of the LD_{50}) by stomach tube. They were then closely

observed for a period of seven days. At 6, 12, 24, 48, 72 and 96 hours post dosing 5 rats were anesthetized and their blood sampled, they were then sacrificed and their brains removed. Plasma, RBC and brain ChE levels were determined.

Results: It was asserted that there was a correlation between the toxic symptoms and the degree of inhibition of the brain and red cell cholinesterase.

SUPPLEMENTARY DATA.

M. Antidotal Study

Three groups of 6 female rats were given a single dose of 3000 mg/kg of undiluted PP 511 by stomach tube. When toxic symptoms appeared, one group was given a subcutaneous injection of 20 mg/kg atropine sulfate, another group an injection of a mixture of 20 mg/kg atropine and 50 mg/kg P-25 (pyridmidine - 2 - aldoxime methanesulfonate).

All of the rats receiving no antidote died, only three of the rats receiving atropine died, only one rat receiving the two antidotes died.

Therefore, atropine and the combination atropine and P-25 is antidotal to pirimiphos methyl poisoning.

SUPPLEMENTARY DATA

2. Subacute Toxicity Following Repeated Oral Administration

A group of 10 male and 10 female rats were given 200 mg/kg of P-M, daily by stomach tube, 5 days per week, for 2 weeks.

No toxic signs developed until after the seventh dose. The weight gain of both males and females was severely impaired. Hemoglobin levels were depressed and other blood parameters were altered. Three days after the last dose macroscopic and histopathological examination revealed several lesions, including haemopoieses of the spleen.

The experiment was repeated with 10 male and 10 female rats dosed with 400 mg/kg daily, 5 days a week for 2 weeks. Signs of ChE inhibition were apparent after 2 doses and increased in severity as the experiment progressed. Eventually 9 males and 3 females died.

SUPPLEMENTARY DATA. The maximum tolerated dose is between 200 and 400 mg/kg/day.

3. Subacute Dermal Toxicity

A daily dose of 1000 mg/kg was applied to the dorsal skin of 6 rabbits for 14 days. Contact was for 23 hours with 1 hour between removal and application.

One rabbit died after the 14th application. There were no toxic symptoms until the 6th or 7th application. Most rabbits lost weight.

SUPPLEMENTARY DATA.

4. Vapor Toxicity

4 male and 4 female rats were exposed to a vapor containing 3.5 ppm (saturated and as determined by weight loss) of P-M. Exposure was for 6 hours/day 5 days/week for 3 weeks.

No toxic signs including depression of ChE values were reported as developing. Post mortem examination, both macroscopic and microscopic, did not reveal lesions caused by P-M.

This test is SUPPLEMENTARY. No LC_{50} was determined, an insufficient number of rats were used, no data on particle size or actual concentration of P-M in the atmosphere near the snouts of the rats was reported. Study strongly suggests that pirimiphos-methyl is not a serious inhalation hazard.

5. Sensitization

Application of P-M (50% solution) in olive oil to the ears of 6 guinea pigs for 3 days did not result in erythema when challenge doses of 10%, 5% and 1% of P-M in olive oil were applied to the shaved flank skin 4 days later.

SUPPLEMENTARY DATA. Not consistent with current EPA procedures for a sensitization study.

6. Pirimiphos-methyl PP: 511): Ninety-day oral toxicity study in rats.

Groups of 25M and 25F Alderley Park specific pathogen-free rats were maintained on diets containing 0.0008, 0.008 and 0.36% (8,80 and 360 ppm) for 90 days.

Results

1. All of the animals (except two, a control and high dose) survived. The two rats died of causes not related to the test chemical.

2. Body weight and food consumption were reduced in the females in the 80 and 360 ppm test groups only. The other test groups were comparable with the controls.

3. There were no remarkable abberations in any of the haematological values measured.

4. Cholinesterase Inhibition

   A. Plasma (measured periodically prior to and during the test).

   It was asserted by the testing laboratory that only the 80 and 360 ppm diets resulted in significantly depressed plasma ChE values. However, at 4 weeks, during the dosing period the plasma ChE levels for females fed 8 ppm was 54%. The other values were essentially equivalent to the controls. Males were not inhibited at 8 ppm.

   RBC ChE. There was no consistent depression of RBC ChE at the 8 ppm test level. This enzyme was inhibited at the higher test levels.

   Brain ChE. was inhibited in females receiving the 80 and 360 ppm diets. The males and low-dose females (8 pm) did not show statistically significant depression.

5. There were no dose-related compound induced effects in either gross pathology, histopathology or organ weight changes.

   This test is CORE MINIMIM. Specific blood chemistry tests were not conducted (SGOT etc.).

   NOEL (cholinesterase) is 8 ppm. The lowered plasma ChE for females (at 8 ppm) was considered incidental. The systemic NOEL is also 8 ppm. Females in 80 ppm test group showed depressed weight gain.

7. <u>P 511 Oral Toxicity Study in Dogs (Initial Studies and Related Dosage for thirteen weeks)</u>.

Huntingdon Research Center, March 20, 1970. Study No. 3258/70/70

A preliminary experiment revealed that dogs would not tolerate a dose of 50 mg/kg/day. Therefore the doses of 0, 2.0, 10, and 25 mg/kg/day were selected. 8 Beagle dogs (4F and 4M) was tested at each dose level. The dose was calculated once a week and delivered via a corn oil capsule daily.

Results

1. No deaths resulted.

2. Clinical signs: at 25 mg/kg only there was reported a generalized loss of body condition accompanied by bouts of vomiting and the passage of loose stools (5% of total days).

The passage of loose stools was also above normal for 10 mg/kg (3% of days) vs. controls and 2 mg/kg (< 1% of total days).

3. One dog in the 25 mg/kg dose group acted more sensitive than the other 7.

4. Bodyweight

Significant depressions of bodyweight were reported for dogs receiving the mid- and high-dose levels.

5. Food and Water Consumption

A slight adverse effect in food consumption for dogs fed the high dose levels was noted.

6. Electrocardiography-

A depression ($p = .05$) in heart rate (144 bpm vs. 156 bpm) was noted in the high test group only.

Laboratory Investigations

Cholinesterase (brain, plasma and erythrocyte)

A. Plasma levels. Mild depression at all levels. At 2 mg/kg, 20% depression was noted. This may not be biologically significant.

B. Erythrocyte levels.

Slight depression in the group receiving 2 mg/kg/day was observed after 10 weeks of dosing. At the high doses the inhibition was progressive. At the lowest level depression of RBC ChE reached 21% and 26% and was statistically highly significant.

C. Brain ChE

No significant differences in brain ChE were reported. The post mortem to assay time is not stated, thus these data are questionable.

2. Other Clinical Laboratory Investigations

All biochemical, hematological and urinalysis values were within normal limits at the initiation of this study.

After 3, 6 and 13 weeks of dosing, the haematological and urinalysis values were within normal limits.

SGPT levels were evaluated for dogs receiving 10 or 25 mg/kg but not the dogs receiving 2 mg/kg (The data for 6 and 13 weeks are tabulated, statements concerning the data for 3 weeks were made, but the data could not be found).

Not all dogs at the high doses demonstrated this adverse effect. In particular only 2 males in the high dose group and 1 male at the mid-dose group consistently (at 6 and 13 weeks) showed this SGPT effect. The dogs that showed this adverse effect also showed an increase in serum alkaline phosphatase indicating liver damage.

Terminal Studies

2M and 2F from each group were sacrificed and the others allowed to recover and then sacrificed.

1. The dogs with high SGPT levels were found to have liver damage. Other incidental abnormalities were also reported.

2. The brain to bodyweight was increased in the high-dose group, but this was attributed to loss in weight.

3. No dose-dependent lesions were observed histopathologically although one dog receiving the mid-dose and one dog receiving the high dose showed bile duct proliferation.

This test is CORE MINIMUM. A NOEL of <2 mg/kg/day (cholinesterase effects) is noted. Highly significant RBC ChE inhibition occurs at this level. Systemic NOEL = 2 mg/kg/day.

The problem of hepatic changes induced by pirimiphos methyl was further examined in a subsequent study.

8. Pirimiphos Methyl (PP 511): A Study of Hepatic Changes in the Dog.

Report CTL/P/283, (Central Toxicology Laboratory of ICI, Study dated, October 1976.

A previous 90-day subacute study in dogs revealed that at a dose level of 25 mg/kg/day mild hepatic effects characterized by minimal bile duct proliferation and associated elevated levels of plasma alanine amino transferase and alkaline phosphatase activity were evident. The purpose of this study was to investigate in more detail the potential of pirimiphos-methyl to cause hepatic changes in the dog.

16 beagle dogs from the Alderley Park strain were grouped as 6 controls and 10 test animals. The test animals were treated with 25 mg/kg/day of pirimiphos-methyl. Two of the treated dogs were given increased doses after the 8th week (35 mg/kg) and later 45 mg/kg and then 50 mg/kg/day. The dose was administered as a gelatine capsule in corn oil.

Results

1. Clinical Observations

Several dogs receiving P-M showed pronounced clinical effects which included loss of appetite, loss of bodyweight, vomiting, diarrhea, soft feces, behavioral abnormalities and other symptoms of ChE inhibition. Four dogs were sacrificed in extremis.

2. Both food consumption and bodyweight changes were noted in the treated dogs.

3. Clinical Biochemistry (determined weekly). plasma alkaline phosphotase, ALP, plasma ornithine carbamyl transferase, OCT., plasma aspartate amino transferase, plasma glutamate dehydrogenase, plasma alanine aminotransferase.

Definite elevations in plasma levels of one or more of these enzymes were noted in most dogs, some also showing concurrent increases in clinical effects (as above).

4. Pathology

i) only two dogs showed liver pathology, or cases of focal and diffuse discoloration.

ii) Gastrointestinal tract - several assorted discolorations, areas of erosion and congestion. The peritoneal cavity held a clear fluid. (unspecified).

iii) microscopic findings revealed slight focal inflammation. A minimum degree of bile duct proliferation was present in two dogs.

iv) The gastrointestinal tract also revealed slight focal inflammation in the mucosa of two dogs. In one dog severe ulceration with inflammatory cell infiltration was present.

Discussion

The liver and gastrointestinal damage induced by P-M is not consistent but appears to be related to P-M in the diet.

CORE MINIMUM data, No NOEL for these effects is established in this experiment.

9. FP 511: Oral Toxicity Study in Beagle Dogs (2 year)
Huntingdon Research Centre, Huntingdon, England. May 8, 1973.

4 groups of 4M and 4F pure-bred beagle dogs were dosed with 0, 0.5, 2.0 or 10.0 mg/kg/day once a day, 7 days a week for two years. The pesticide was dissolved in corn oil, placed in a gelatine capsule and the dogs were dosed orally.

Results:

1. One dog (a female fed 10 mg/kg/day) died on the 401 day. Its death could not be positively related to the test chemical.

2. Clinical signs. There were increases in loose stools at 2 and 10 mg/kg/day. This could have been a chemically related effect.

3. Minor decreases in bodyweight gain and food consumption were noted in the higher test levels. Water consumption was unaffected.

4. Periodic examination of the dogs' eyes and electrocardiography did not reveal a chemically related effect.

5. Cholinestrase Inhibitions.

   Plasma and RBC Cholinesterase values were determined both before the start of the test diet, during the 2-year test (periodically) and after sacrifice. Brain levels were determined after sacrifice.

   The test laboratory asserts that significant decreases in all three sources of ChE were evident at 2.0 and 10 mg/kg. Their assertion includes the ChE depression was not of biological significance at the 0.5 mg/kg/day.

   At the 0.5 mg/kg/day during the treatment period the plasma ChE was around 20% below the control level (possibly biologically significant). The RBC ChE was essentially equivalent to the control. Brain ChE was 19% below the control level and this value was statistically significant (p of 1%).

   TOXICOLOGY BRANCH has decided that the NOEL for ChE is < 0.5 mg/kg/day based on the brain ChE data. This conclusion is obscured by the notation that the dogs were sacrificed over a week's time after the diet was stopped. It is possible that even greater depressions in brain ChE were realized that would have been detected if the brain tissue was assayed immediately after sacrifice.

   The results of haematological analysis, biochemical measurements and urinalysis were within normal limits for this breed of beagle dog.

6. Gross Pathology. There were no compound related adverse effects noted.

7. Organ weights - It was asserted that no compound-related changes, dose-dependent changes were noted although the liver weight at the highest dose was higher than the controls. Male gonads (testis) were of lesser weight for the two highest doses.

8. No consistent dose dependent histopathological lesions were noted for the test animals.

This test is CORE GUIDELINES. However, no NOEL was established for cholinesterase effects (brain). The systemic NOEL is = 2 mg/kg/day.

10. Long-term feeding of Pirimiphos-Methyl (PP-511) in mice. (Oncogenicity)

ICI 3417658, dated July 15, 1976.

Pirimiphos-methyl (P-M) was tested in an 80-week chronic feeding study in mice (CFLP) as follows:

	No. of Mice		Dose (mg/kg/day)	
Dose	M	F	M	F
0 ppm	64	64	0	0
5 ppm	64	64	0.5	0.6
250 ppm	52	52	25.9	27.6
500 ppm	64	64	45.0	50.6

All groups except the 250-ppm group contained 12 extras for ChE determination. The 500-ppm group initially started on a diet containing 300 ppm and was increased weekly by 50 ppm to 500 ppm.

P-M was dissolved in corn oil and mixed with Spratt's laboratory animal diet at 1000 ppm. This stock diet was prepared twice weekly for the first 63 weeks and then weekly thereafter. The P-M in the diet was determined at regular intervals throughout the study by a gas chromatographic method.

Results

1) Clinical Signs. No overt signs of reaction were reported.

2) Mortality and survival. No dose-dependent mortality was evident. Decedents were stated as being similiar in pathology for controls and test animals.

3) No definite dose-dependent or compound-related effects were noted relative to body weight gain, food consumption, food efficiency, or water consumption.

4) Cholinesterase activity (groups 0, 5 ppm and 500 ppm)

   a) RBC ChE - Not considered of biological significance (<25%) at 5 ppm.

b) Plasma, ChE - Only borderline changes were reported as occurring at 5 ppm. These were statistically highly significant and 10 to 35% lower than controls.

c) At 500 ppm, marked and consistent RBC and plasma ChE inhibition were noted.

Terminal Studies

There was no evidence presented to indicate that P-M altered the spontaneous tumor profile of the CFLP mouse. This conclusion was derived after an apparently comprehensive and inclusive examination both macroscopically and histologically.

This test is CORE MINIMUM. No evidence of compound-related tumors is presented or demonstrated. The NOEL (AChE inhibition) is 5 ppm. There were slight but questionably biologically significant effects at 5 ppm.

As a chronic feeding study this test is CORE SUPPLEMENTARY. Many other tests and evaluation should have been made.

11. Pirimiphos-methyl (PP-511): 2-year feeding study in the rat

Report No: HO/IH/P/113, dated June 1974.

Groups of 48 male and female Wistar-derived rats were fed 0, 10, 50, or 300 ppm pirimiphos-methyl for two years. Additional groups of 24 rats at each dose level for each sex were fed the same levels and sacrificed at interim periods up to one year. This study was designed to be a chronic feeding and oncogenesis study.

Results

1) Clinical Conditions: There were two transient outbreaks of disease, but this did not appear to upset the experiment since both controls and test animals were equally affected.

2) Mortalities: There was no dose-dependent mortality trend.

3) There were no consistent dose-dependent adverse effects on body weight, food consumption or food utilization that were remarkable.

4) Haematological measurements were made at 26, 52, 67 and 87 weeks to determine haemoglobin, packed cell volume, mean corpuscular

haemoglobin concentration, white cell counts, differential, reticulocytes, platelets and mean corpuscular diameter and clotting functions.

There were no consistent dose-dependent adverse effects noted except for a slight anemia in the high-dose females.

5) Cholinesterase Inhibition (Pirimiphos-methyl is an organophosphate cholinesterase inhibitor)

A. RBC Cholinesterase

Neither 10 nor 50 ppm pirimiphos-methyl consistently affected RBC cholinesterase in either males or females.

B. Plasma Cholinesterase

In males there was no noticeable effect at 10 ppm; at 50 ppm, from the middle of the feeding program onward there was some inhibition (ca.40%).

In females, at 10 ppm the plasma ChE was usually 8 to 24% below the controls. AT 50 and 300 ppm there was constant inhibition.

ChE determinations were made weeks 1, 2, 3, 4, and 5 preexperiment and weeks, 2, 4, 6, 8, 12, 26, 37, 52, 65, 78, 91 and 104 during the experiment and weeks 1, 4 and 8 recovery period.

6) There were no dose-dependent compound-related changes in organ weight or organ:body weight ratios. (Liver, kidneys, adrenals, heart, lungs spleen) reported.

7) Pathology reports (macroscopic and histological) were included. There were no dose-dependent, compound-related increases in tumors noted.

Conclusion: This test is CORE MINIMUM. Not all blood chemistry determinations were made, no urinalysis was made.

The data support a NOEL of 10 ppm. At higher levels there is inhibition of plasma cholinesterase. No evidence that pirimiphos-methyl induces or promotes tumors was evident. The systemic NOEL is = or less than 300 ppm. There was a slight anemia noted in females at this dose level.

12. Dominant Lethal Study in mice of Pirimiphos-Methyl

Inveresk Research International, Project No. 402864, dated June 20th, 1975. (D. B. McGregor)

Five groups of 15 male mice of proven fertility (CD-1 strain) were treated immediately before test mating at dose levels of 15, 80, or 150 mg/kg of P-M in corn oil. Positive control groups comprised 100 mg/kg of ethyl methanesulfonate (in water) and 200 mg cyclophosphamide (in water). The control group (30 mice) were treated with corn oil.

Following treatment, the male mice were housed with two females. After seven days, the male mice were transferred to fresh cages and mated with a second batch of female mice. This process was repeated until the treated male mice had been mated at weekly intervals for eight weeks with virgin females. The males were sacrificed after the last mating, and the different batches of females were sacrificed 13 days after the assumed date of fertilization.

The highest dose tested for P-M was 150 mg/kg and this dose was close to the five-day oral acute LD_{50} (200 mg/kg).

In this test P-M did not cause increases in the percentage of early deaths, or number of early deaths per pregnancy, or preimplantation losses. There was an overall decrease in fertility in the high-dose group. The positive controls gave positive responses.

This test is CORE MINIMUM. At the levels tested, P-M does not induce chromosomal abnormalities.

13. Mutagenicity of Organophosphorous Compounds in Bacteria and Drosophila

Mutation Research 28: 405-420
P. J. Hanna and K. F. Dyer
Dept. of Genetics
Monash University, Clayton, Victoria 3165 (AUSTRALIA)

P-M is included among compounds which give a positive response in testor strains of Salmonella typhimurium, notably TA 1530 and TA 1535, when 5-10 µl (?) of the test chemical per plate are assayed.

These data are SUPPLEMENTARY. This demonstration of a positive mutagenic effect must be further studied. Since P-M was one of 140 chemicals tested, TOXICOLOGY BRANCH cannot accept the test as

conclusive evidence that P-M is mutagenic. A second test comparing only P-M with known positive controls must be presented. In addition, both activation and nonactivation tests should be run. The submitted test does not adequately define the amount used per assay.

Examination of Pirimiphos-methyl for neurotoxicity in the Domestic Hen.

Huntingdon Research Centre, ICI/49/75220, August 11, 1975 (Amended Dec. 19, 1978).

Seven groups of five birds were grouped and dosed as follows:

Group	No. Birds	Dose Rate (mg/kg)	Mortality	Birds Added
1	5	0	0	
2	5	(500 ppm TOCP)	-	
3	5	20	0	
4	5	30	0	
5	5	40	1	
6	5	50	1	+1 50
7	5	60	3	+3 60
		Part A (21 days)	5-day interval	Part B (21 days)

From Part A of this experiment, an LC_{50} for hens of 79 mg/kg was calculated, based on mortality after 21 days.

The birds surviving the doses of pirimiphos-methyl of 50 and 60 mg/kg were redosed and protected with atropine and PAM; three additional birds were added to the 60 mg/kg group and one additional bird was added to the 50 mg/kg group.

Results

1) Bird weight was not noted to be seriously affected by pirimiphos-methyl. TOCP-treated birds lost weight.

2) Clinical assessment

   a) Three of the 60 mg/kg pirimiphos-methyl birds died after redosing. One of the 50 mg/kg pirimiphos-methyl birds died.

3) There was some (lowest degree) evidence of incoordination in two birds receiving the 50 mg/kg dose and in one bird receiving the 60 mg/kg dose. These were not considered by the testing laboratory as being symptoms of delayed neurotoxicity.

4) Histology. Only two of the birds treated with pirimiphos-methyl were examined histologically. It was stated that minimal changes were evident (cuffing with mononuclear cells) but no evidence of axonal degeneration was stated.

This test is CORE SUPPLEMENTARY. An insufficient number of chickens was used. Those surviving the first LD_{50} dose (doses used were less than the LD_{50}) should be treated with a second LD_{50} dose and all birds should be examined histopathologically.

14. Pirimiphos-methyl (PP 511): Teratogenicity Study in the Rabbit.

ICI Central Toxicology Laboratory, HO/CTL/P/119B, July 1974

Forty-nine virgin Dutch rabbits, supplied by Cheshire Rabbit Farm, Ltd., Tarporley, Cheshire, were used in this experiment. Six males were used for stud service. Six does were mated daily over a period of two weeks with untreated proven bucks. Following the mating period, each doe received 25 i.u. of chorionic gonadotrophin in order to promote ovulation. The day of mating was termed day 0 and the rabbits were dosed with P-M on days 1 to 28 inclusive. The rabbits were treated with gelatin capsules containing doses of either 0, 1 or 16 mg/kg of P-M. On the 29th day the rabbits were sacrificed and the foetuses were removed immediately and preserved for examination.

Results: 10 controls, 10 low-dose and 11 high-dose group rabbits became pregnant and their pups were subsequently examined for defects.

The only observable adverse effect in the high-dose group dams was depression of both plasma and RBC ChE. Body weight was not affected.

Only one grossly abnormal fetus was reported in the high-dose (16 mg/kg) group. Only one abnormality (in a different animal) was noted in fixed tissues.

Lower foetal weights for both the 1 mg/kg and 16 mg/kg groups were stated. But this was attributed to the increased litter size. The numbers of absorptions was the same in all groups.

The ratio of male to female pups showed a dose-dependent increase (1.0, 1.25 and 1.39), but this was stated as being within normal variation.

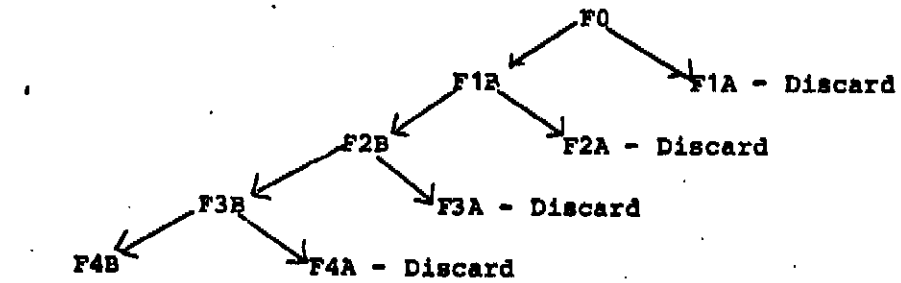
This test is SUPPLEMENTARY. At least three dosage levels should have been included; a positive control should have been included. The highest dose should produce effects in the dam more serious than the ChE depression. The pups should also show some signs of fetotoxicity. At least 12 pregnant rabbits should be used per dose level.

This test is upgraded to CORE MINIMUM. In spite of the deficiencies listed above, the 16 mg/kg dose is reasonably above what might be expected from residues and there were no signs of teratology or fetotoxicity at this dose. However, a teratology study in a second species will be required for permanent tolerances.

15. Effects of PP 511 (Pirimiphos-methyl) on reproductive function of multiple generations in the rat.

Huntingdon Research Center, Dec. 7, 1972, 5457/72/853

Three groups of rats (CD strain, Charles River) each consisting of 12 M and 24 F were fed 0, 20 or 200 ppm of P-M in their diets. These rats (F_0) were mated, and 12 M and 24 F weanlings were selected to produce F_1B generation. The process was extended to produce a F_2B generation and succeeding generations. For example:



Results

1) There were no overt signs of malreaction to treatment in any generation. Body weight and food consumption were the same for treated and control groups.

2) Mating performance (males) was impaired at the 200 ppm from the F1B generation onward. At 20 ppm there were observable impairments at the F1B and F2B generations, but the F3B generation was essentially comparable to controls. (Note: mating performance as assessed by the number of males successfully impregnating both available females.

3) In females, a dosage-related reduction in pregnancy rate that was in some cases statistically significant. For example at 200 ppm, the F1B generation, and the F2B generation at 20 and 200 ppm. The gestation period was essentially comparable for all groups throughout the three generations.

4) Terminal autopsies of parent animals (F0, F1B, F2B and F3B generations) were performed and it is asserted that no dose-dependent, or compound-related lesions were evident.

5) No consistent differences in litter size, litter and mean pup weights, pup mortality or pup abnormalities were reported, although some differences in pup weight were evident at 200 ppm.

6) The ultimate generation of the controls and 20 ppm treated groups and the controls, 20 and 200 ppm groups of the F3 generation were subjected to macroscopic organ weight analysis, skeletal examination and histopathological examination.

   a) At 200 ppm, statistically significant differences in male heart and kidney weights were evident. These corresponded to other differences in lower mean body weight. At 20 ppm, the F4B generation only the relative kidney weight for males differed from the control.

   b) Skeletal differences were lower than (200 ppm) or comparable with (20 ppm) controls.

   c) No dose-related histopathological abnormalities were reported.

This test is CORE MINIMUM. Adverse effects on reproduction were noted at 200 ppm. The impairment at 20 ppm for male performance was statistically significant for the F2B generation (second mating), but the subsequent matings were apparently normal. This test does not firmly establish a NOEL.

Appendix

Effect of PP 511 (Pirimiphos-methyl) on reproductive function of multiple generations in the rat: Histopathological examination of testis of F1B and F2B males.

Reduced mating performance and pregnancy rate had been noted for animals of the F1B and F2B generations at the 200 ppm level diet, thus histopathological examination of the adult males was undertaken in an effort to explain these findings.

Sections of testis stained with haematoxylin and eosin were examined from the following groups of rats: Control of F1B and F2B generation and 200 ppm of the F1B and F2B generation.

The degree of activity and maturity of the process of spermatogenesis was found to be comparable for both the control and treated rats.

16. Effect of Pirimiphos-methyl (PP 511) on reproductive function of multiple generations in the rat

Huntingdon Research Centre, ICI 63/76534, 31 August 1976

Specific pathogen-free rats of the CFY strain were grouped (20 M and 20 F) and fed diets containing 0, 5, 10 or 100 ppm of P-M. The animals were housed by sex, five to a cage. During the mating period, the rats were paired (one to one); the females were subsequently housed in individual cages until their pups were weaned.

F0, F1, F2 and F3 generations were produced. All pups were examined for gross abnormalities. Selected pups were examined for weight differences.

Results

1) There were no signs of malreaction due to P-M. Diet, body weight change and food consumption were asserted to be the same for control and treated groups.

2) Some blood ChE depression resulted for the rats treated with 100 ppm of P-M. Female plasma ChE was consistently inhibited at 100 ppm, but males were unaffected. At lower levels only occasional plasma ChE depression was noted. RBC ChE was depressed for both males and females at 100 ppm, but these depressions did not exceed 20% decline.

3) Mating performance was asserted to be essentially the same for all groups.

4) Gestation was essentially comparable for all groups, terminal autopsy of parent animals revealed no macroscopic changes attributable to treatment.

Litter Data:

1) There was a high incidence of litter loss that appeared to be P-M related for the F0 generation, but this was not evident for the F1 and F2 generations.

2) There were no statistically significant, intergroup differences in litter size and pup mortality, or litter pup weight. There were no treatment-related abnormalities in any generation.

3) The terminal examinations of the F3 generation revealed only higher liver weights in the 10 ppm group and lower thyroid weights in the 100 ppm group. However, these values were not statistically significant after adjustment for body weight.

This test is CORE MINIMUM. The test should have been designed so that mating always gave 20 females that produced litters. This test does not demonstrate that the decrease in mating performance noted at 200 ppm also occurs at 100 ppm.

17. Erythrocyte and Plasma Cholinesterase Activity in Human Volunteers Administered Pirimiphos-methyl (Interim Report)

ICI Report No. HO/CTL/P/128, August 1974

Five healthy male adults 59.5-73 kg body weight and 25-45 years of age volunteered for this study. They were given a dose of 0.25 mg/kg per day for a total of 28 days (416.5 to 511 mg. total). The dose was taken orally by capsule at mid-morning of each day. Blood samples were also taken at mid-morning on days 1, 3, 7, 14, 21, 28, 35, and 42 days.

Both plasma and erythrocyte ChE activities were within normal values for healthy males. Thus this dose level of P-M has no effects on these enzymes.

This test provides SUPPLEMENTAL DATA. (There are no EPA guidelines.)

18. The human response to long-term oral administration of low doses of Pirimiphos-methyl

ICI, Plant Protection Division, October 30, 1976

Three male [illegible] three female volunteers were given oral doses (in a gelatin capsule) of 0.25 mg/kg/day of P-M for a period of 56 days. Two additional females received blank capsules over the same period.

The following tests were performed ChE (erythrocyte and plasma, plasma alanine aminotransferase, plasma aspartate aminotransferase, plasma - glutamyl transpeptidase, plasma alkaline phospatase and blood counts.

Samples of blood were taken prior to, during and following the administration of test chemical.

Results

Some slight plasma ChE changes were noted, but all other parameters were within normal limits.

This test provides SUPPLEMENTARY data.

19. Acute toxicity of metabolites

The following metabolites of Pirimiphos-Methyl were assayed for their Acute oral LD_{50}.

	Compound	Testing Lab.	No. of Rats per Test Group	LD_{50}
1)	2-diethylamino-4-hydroxy-6 methylpyrimidine	ICI	3	800-1600 mg/kg
2)	2-amino-4-hydroxy-6-methyl-pyrimidine	ICI	6	>4000
3)	2-ethylamino-4-hydroxy-6-methyl pyrimidine	ICI	6	2090 (1841-2330,
4)	dimethyl 2-ethylamino-4-methyl-pyrimidin-6-yl phosphorothionate	ICI	3	800-1600
5)	ethyl guanidine	ICI	6	706 (634-786)
6)	diethyl guanidine	ICI	6	545 (470-625)
7)	Guanidine hydrochloride	ICI	6	1105 (1000-1220)

8)	Methyl guanidine sulphate	ICI	6	1105 (950-1285)
9)	Dimethyl guanidine hydrochloride	ICI	6	1445 (1215-1720)

All of the above studies provide SUPPLEMENTAL DATA. Only one sex was tested, necropsy was not performed, behavioral reactions were not monitored.

Execretion, Metabolism and Retention

Several studies were submitted examining the excretion, metabolism and retention of ^{14}C pirimiphos-methyl in rats, dogs, cows, goats, and chickens.

These studies demonstrated that pirimiphos methyl is rapidly absorbed from the gastrointestinal tract, excreted in the urine and nearly all of the isotope is eliminated within 96 hours. The studies also demonstrated that small amounts could be transferred to milk and eggs. However, the amount transferred is small and maintained by the petitioner as not toxicologically significant.

Some attempts to identify the chemical structures of the metabolites were presented, but only comparative methods were used and these were not confirmed by GC-MS.

TOXICOLOGY BRANCH defers to CHEMISTRY BRANCH to determine the extent of residues in milk and eggs and the structure of the metabolites.

8-point memo (PP 9G2154 and FAP 9H5201)

1. Toxicological data considered in setting this tolerance include:

   a). adequate acute toxicity data to justify a "WARNING" signal word on the label.

   b). subacute and chronic studies

90-day rat feeding	NOEL = 8 ppm (ChE and systemic)
90-day dog feeding	NOEL < 2 mg/kg/day (ChE) NOEL = 2 mg/kg/day (systemic)
mouse oncogenesis (oral feeding)	Negative for oncogenic effect NOEL = 5 ppm (ChE)
2-year rat chronic feeding/ oncogenesis	NOEL = 10 ppm (ChE) NOEL = 300 ppm (systemic) Negative for oncogenic effect
2-year Dog chronic feeding	NOEL < 0.5 mg/kg/day (ChE) NOEL = 2 mg/kg/day (systemic)
Teratology - rabbits	Negative for terata at 16 mg/kg/day
Reproduction - rats	NOEL = 100 ppm.
Neurotoxicity - hens	No neurotoxic effect demonstrated

2. Data considered desirable but currently lacking:

   a. repeat of the acute delayed neurotoxicity study in hens.
   b. mutagenesis study (Ames test).

3. EPA has requested (in this review) that these studies be initiated.

4. There are no established permanent tolerances for this chemical.

5. Granting this tolerance will raise the percent ADI from 0 to 2.92%.

6. The TMRC is 0.087 mg/day/1.5 kg. The MPI is 3.000 mg/day/60 kg. The rat 2-year feeding study with a NOEL of 10 ppm (ChE inhibition) and a safety factor of 10 were used to calculate the ADI (0.05 mg/kg/day).

7. Toxicology Branch has no knowledge of pending regulatory actions against registration of pirimiphos-methyl.

8. None.

3/16/80

END